Improved diagnostics help to identify clinical features and biomarkers that predict Mycoplasma pneumoniae community-acquired pneumonia in children

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Abstract: BACKGROUND There are no reliable signs or symptoms that differentiate Mycoplasma pneumoniae (Mp) infection in community-acquired pneumonia (CAP) from other etiologies. Additionally, current diagnostic tests do not reliably distinguish between Mp infection and carriage. We previously determined that the measurement of Mp-specific IgM antibody-secreting cells (ASCs) by enzyme-linked immunospot (ELISpot) assay allowed for differentiation between infection and carriage. Using this new diagnostic test, we aimed to identify clinical and laboratory features associated with Mp infection. METHODS This is a prospective cohort study of children, 3-18 years, with CAP from 2016-2017. Clinical features and biomarkers were compared between Mp-positive and -negative groups by Mann-Whitney U test or Fisher's exact test, as appropriate. Area under the receiver operating characteristics curves (AUC) differences and optimal thresholds were determined by using the DeLong's test and Youden's J statistic, respectively. RESULTS Out of 63 CAP patients, there were 29 Mp-positive (46%). Mp-positive was statistically associated with older age (median 8.6 vs. 4.7 years), no underlying disease, family with respiratory symptoms, prior antibiotic treatment, prolonged prodromal respiratory symptoms and fever, and extrapulmonary (skin) manifestations. Lower levels of C-reactive protein, white blood cell count, absolute neutrophil count, and procalcitonin (PCT), specifically PCT <0.25 ug/L, were statistically associated with Mp infection. A combination of age >5 years (AUC=0.77), prodromal fever and respiratory symptoms >6 days (AUC=0.79), and PCT <0.25 g/L (AUC=0.81) improved diagnostic performance (AUC=0.90, p=0.05). CONCLUSIONS A combination of clinical features and biomarkers may aid physicians in identifying patients at high risk for Mp CAP.

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Improved diagnostics help to identify clinical features and biomarkers that predict *Mycoplasma pneumoniae* community-acquired pneumonia in children

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Summary: Community-acquired pneumonia (CAP) caused by *Mycoplasma pneumoniae* (*Mp*) is difficult to diagnose. A combination of clinical features and biomarkers may aid physicians in identifying patients at high risk for *Mp* CAP.

Abstract

Background: There are no reliable signs or symptoms that differentiate *Mycoplasma pneumoniae* (*Mp*) infection in community-acquired pneumonia (CAP) from other etiologies. Additionally, current diagnostic tests do not reliably distinguish between *Mp* infection and carriage. We previously determined that the measurement of *Mp*-specific IgM antibody-secreting cells (ASCs) by enzyme-linked immunospot (ELISpot) assay allowed for differentiation between infection and carriage. Using this new diagnostic test, we aimed to identify clinical and laboratory features associated with *Mp* infection.

Methods: This is a prospective cohort study of children, 3–18 years, with CAP from 2016–2017. Clinical features and biomarkers were compared between *Mp*-positive and -negative groups by Mann-Whitney *U* test or Fisher's exact test, as appropriate. Area under the receiver operating characteristics curves (AUC) differences and optimal thresholds were determined by using the DeLong's test and Youden's J statistic, respectively.

Results: Out of 63 CAP patients, there were 29 *Mp*-positive (46%). *Mp*-positive was statistically associated with older age (median 8.6 vs. 4.7 years), no underlying disease, family with respiratory symptoms, prior antibiotic treatment, prolonged prodromal respiratory symptoms and fever, and extrapulmonary (skin) manifestations. Lower levels of C-reactive protein, white blood cell count, absolute neutrophil count, and procalcitonin (PCT), specifically PCT <0.25 ug/L, were statistically associated with *Mp* infection. A combination of age >5 years (AUC=0.77), prodromal fever and respiratory symptoms >6 days (AUC=0.79), and PCT <0.25 µg/L (AUC=0.81) improved diagnostic performance (AUC=0.90, *p*=0.05).

Conclusions: A combination of clinical features and biomarkers may aid physicians in identifying patients at high risk for *Mp* CAP.

Keywords: antibiotics; C-reactive protein; diagnosis; procalcitonin; treatment

Introduction

Mycoplasma pneumoniae (*Mp*) is a common bacterial cause of community-acquired pneumonia (CAP) in children [1, 2]. There are substantial challenges in differentiating infection from carriage for *Mp* [3, 4]. Current diagnostic tests, including polymerase chain reaction (PCR) of upper respiratory tract (URT) specimens or serology, do not reliably differentiate between *Mp* infection and carriage [4]. Therefore it is not surprising that previous studies found no signs or symptoms to differentiate *Mp* infection in CAP from other etiology [5, 6]. Potentially because *Mp* detection was misclassified as infection when in fact it was carriage. *Mp* carriage is estimated to occur in up to 56% of healthy children [4, 5]. *Mp* infection is generally mild and self-limiting, and patients with *Mp* CAP are mostly managed in primary care [7]. The lack of a cell wall makes *Mp* naturally resistant to first-line empirical β-lactam antibiotics for CAP [5]. Empirical macrolide treatment is extensively used to cover potential *Mp* infection, which has led to the emergence of macrolide-resistant *Mp* and a parallel rise in macrolide resistance in other respiratory pathogens [8]. Therefore, with the challenges in diagnostic testing for *Mp* and it's limited use in the primary care setting, it is essential to determine clinical features and biomarkers to aid in the diagnosis of *Mp* infection in children with CAP.

We recently demonstrated in a prospective cohort study of CAP in children that the measurement of specific peripheral blood immunoglobulin (Ig) M antibody-secreting cells (ASCs) by enzyme-linked immunospot (ELISpot) assay improves diagnosis of *Mp* infection in CAP [9]. This test differentiated between *Mp* infection and carriage. Using this dataset, we here aimed to identify clinical features and biomarkers associated with *Mp* CAP, in which infection may be more accurately identified with the measurement of *Mp*-specific IgM ASCs.

Methods

Patients

Patients were enrolled between May 1, 2016, and April 30, 2017, at University Children's Hospital Zurich [9]. CAP was clinically defined as the presence of fever >38.5°C and tachypnea according to the British Thoracic Society (BTS) guidelines [2]. Eligible participants were inpatients or outpatients aged 3–18 years. CAP patients <3 years of age were excluded to reduce the probability of viral infection, as it is highest in this age group [10-13]. Patients with hospital-acquired pneumonia, cystic fibrosis or other chronic lung disorders (excluding asthma), or known primary or secondary immunodeficiencies were excluded. The local ethics committee approved the protocol for this study (no. 2016-00148). Written informed consent was obtained from all parents and children ≥14 years of age.

Specimen collection and diagnostic testing

All enrolled patients were investigated for *Mp* in pharyngeal swab samples by specific real-time PCR [9, 14]. If additional consent was given, blood samples were collected for separation of peripheral blood mononuclear cells (PBMCs) and serum [9]. Serum was stored at –80°C. As detailed elsewhere [9], only CAP patients were included in this study if fresh (isolated ≤4h) PBMCs were available to avoid poor ELISpot assay performance, and tested for the presence of *Mp* IgM ASCs [9]. A CAP patient with a positive *Mp* IgM ASC ELISpot assay result was considered to have CAP caused by *Mp* (*Mp*-positive). If *Mp* IgM ASCs were not detected, the patient was considered to have CAP caused by another etiology (*Mp*-negative). We additionally investigated the frequency of *Mp* by PCR from pharyngeal swabs among household contacts available for sampling at presentation of index patients.

After study closure, pharyngeal swab samples kept at –80°C were additionally tested for *Streptococcus pneumoniae* (*Sp*) by real-time PCR [15], knowing that detection of *Sp* in the URT is likely colonization and not infection [16]. In fact, co-infection with *Sp* and *Mp* is uncommon, whereas co-colonization may be more common [4, 12]. Another 23 viral and bacterial respiratory pathogens were tested using the ePlex respiratory pathogen panel (RP panel, GenMark Diagnostics, Carlsbad, CA, USA), as previously described [17]. In addition, *Mp* IgM serology was performed (Virion\Serion, Würzburg, Germany). The study test results were not available to treating clinicians.

Clinical data

Demographic, epidemiological, and clinical data were systematically collected using a standardized questionnaire. Full recovery was assessed until 6 months after enrollment. Chest radiographs were ordered for clinical reasons and therefore were not available in three (5%) patients. Chest radiographs were assessed by a radiologist during routine clinical care. These radiological findings and the corresponding images were retrospectively reviewed by two of the authors, who were blinded to clinical information, using criteria for radiographic pneumonia [18, 19].

Laboratory data

Blood cell count and C-reactive protein (CRP) analysis was performed as part of routine clinical care. Procalcitonin (PCT) testing was performed retrospectively in a batched analysis and results were not available to treating physicians. PCT levels were measured using a sensitive assay with a detection limit of 0.007 ng/mL (B·R·A·H·M·S PCT sensitive KRYPTOR, Thermo-Scientific, Berlin, Germany) [20].

Statistical analysis

The non-parametric Mann–Whitney *U* test was used to compare continuous variables and the Fisher's exact test to compare proportions between *Mp*-positive and *Mp*-negative groups. We calculated the area under the receiver operating characteristics (ROC) curves (AUC) of clinical features and biomarkers in differentiating between groups determined by *Mp* IgM ASC ELISpot assay (reference standard). We considered AUC≥0.75 as adequate discrimination [21]. AUC differences were calculated using the DeLong's test. Missing values were removed (ignored by roc.formula). The best biomarker threshold was defined as the optimal cut-off that maximized the distance to the identity (diagonal) line in the ROC curve according to Youden's J statistic. All reported *p*-values are 2-tailed with statistical significance defined as *p*-value <0.05. Data were analyzed using the R software environment, version 3.6.0 [22].

Results

Study population

During the 12-month study period, 152 CAP patients were enrolled and *Mp* DNA was detected by PCR in 44 (29%) participants. Of the enrolled population, 63 (41%) CAP patients met the criteria of having fresh PBMCs available to undergo diagnostic testing with the *Mp* IgM ASC ELISpot assay and were included in this study. Of these, 29 (46%) were *Mp*-positive, determined by detection of *Mp* IgM ASCs. As detailed elsewhere [9], all 29 *Mp* IgM ASC-positive patients were also *Mp* PCR-positive and IgM-positive. However, *Mp* PCR was also positive in three (5%) patients, who were *Mp* IgM ASC- and IgM-negative, and *Mp* IgM found in another three (5%) patients, who were *Mp* IgM ASC- and PCR-negative. Chest radiographs were

performed in 60 out of 63 (95%) included CAP patients, whereof 59 (98%) met the criteria for radiographic pneumonia.

Clinical characteristics

The median age of *Mp*-positive patients (8.6 years, interquartile range [IQR] 6.3–11.0) was higher compared to *Mp*-negative patients with CAP (4.7 years, IQR 3.9–6.2, $p<0.01$) **(Table 1)**. *Mp*-positive CAP was more likely than *Mp*-negative CAP to present during summer (odds ratio [OR] 3.3, confidence interval [CI] 0.8–16.8). Underlying disease was less common among *Mp*-positive than *Mp*-negative patients (3% vs. 26%, $p=0.02$). *Mp*-positive patients were significantly more likely than *Mp*-negative patients to have received prior antibiotic treatment (OR 4.6, CI 1.3–19.6), whereas amoxicillin with or without clavulanic acid (co-/amoxicillin) was the most frequent agent in both groups (*Mp*-positive, $n=9/13$, 69% vs. *Mp*-negative, $n=4/5$, 80%). A positive family history for respiratory tract infection (RTI) was reported in 17 (59%) *Mp*-positive compared to 10 (29%) *Mp*-negative patients (OR 3.3, CI 1.1–11.0, $p=0.02$). Household contacts of *Mp*-positive index patients were significantly more likely to have *Mp* detected in the URT than those of *Mp*-negative index patients (OR 9.7, CI 2.0–93.2, $p<0.01$). Only two family members (5%, both mothers) of *Mp*-negative index patients were PCR-positive, of which one had RTI symptoms. Among household contacts of *Mp*-positive index patients, *Mp* was detected in the URT of nine siblings (64%), seven mothers (35%), and one father (8%). Most of them reported having RTI symptoms (59%), but six siblings and one mother were asymptomatic carriers.

The duration of RTI symptoms and fever prior to CAP diagnosis was longer in *Mp*-positive (median 9.0 days, IQR 6.0–10.0) than *Mp*-negative patients (4.0 days, 3.0–

7.0, $p<0.01$). No other symptoms and signs were statistically different between groups. *Mp*-positive patients were no different than *Mp*-negative patients to have consolidation, interstitial infiltrates, or pleural effusion. Extrapulmonary manifestations were only observed in *Mp*-positive children with CAP (31% vs. 0%, $p<0.01$), and included dermatological and neurological disorders **(Table 1)**. After inclusion, nine (31%) of *Mp*-positive patients were not treated with an antibiotic *in vitro* active against *Mp*.

Hospitalization rates and length of hospital stay (LOS) were similar between the two groups. Detailed characteristics for hospitalized and ambulatory *Mp*-positive and *Mp*-negative patients are shown in **Supplementary Table 1 and 2**. Among hospitalized patients, *Mp*-positive patients were more likely to have oxygen demand, extrapulmonary manifestations, and poor outcome than *Mp*-negative patients. Sequelae were only observed in three (10%) hospitalized *Mp*-positive patients (i.e., bronchiolitis obliterans, exertional dyspnea, and post-inflammatory pigmentary alteration). All *Mp*-negative patients fully recovered.

Laboratory findings

Hematological assessment together with PCT and CRP analysis for *Mp*-positive and *Mp*-negative CAP patients is shown in **Table 2**. *Mp*-positive CAP patients had lower white blood cell count (WBC), absolute neutrophil count (ANC), PCT, and CRP values than *Mp*-negative patients **(Figure 1)**.

Detection of pathogens

Differences in clinical features and biomarkers may be attributed to another pathogen causing a subset of infections. Therefore, pharyngeal swab samples were tested in

this study for other pathogens. Two or more pathogens were found in the URT of 20 (69%) *Mp*-positive and 13 (38%) *Mp*-negative patients **(Table 3)**. *Sp* was equally detected in both cohorts in >60%. Exclusively found in *Mp*-negative patients was respiratory syncytial virus (RSV, *n*=8, 24%, *p*<0.01), influenza virus (*n*=2), parainfluenza virus (*n*=2), and coronavirus (*n*=1). No virus was statistically associated with *Mp*-positive. Rhinovirus, adenovirus, and bocavirus were equally detected in both cohorts. Thus, we also compared clinical features and biomarkers of *Mp*-positive against the following subgroups of *Mp*-negative (*n*=34): first, positive for RSV (*n*=8, as it is the only virus for which detection in the URT has a high predictive value for CAP etiology [16]); second, positive for other viruses than RSV (*n*=7, being aware that URT detection of other pathogens than RSV may not be related to pneumonia [3]); and third, negative for viruses (*n*=19, in which *Sp* was detected in *n*=14 [74%] in the URT and additionally in *n*=2/2 in pleural aspirate during routine clinical care). However, although numbers were again smaller for this subanalysis, *Mp*-positive was also compared to subgroups of *Mp*-negative CAP statistically associated with older age, prolonged prodromal respiratory symptoms, and low levels of PCT **(Supplementary Figure 1 and Supplementary Table 3)**.

ROC analysis and performance curves

ROC analysis was performed for clinical features and biomarkers that were associated with *Mp*-positive CAP. The ROC curves showed good discriminative ability (AUC≥0.75) in differentiating groups for age (AUC=0.77), duration of prodromal symptoms (AUC=0.79), PCT (AUC=0.81), and CRP (AUC=0.76) **(Figure 2A and 2B)**.

The best clinical threshold to discriminate *Mp*-positive from *Mp*-negative was age of 5 years and prodromal fever and RTI symptom duration of 6 days **(Table 4)**. The optimal biomarker thresholds were 0.25 µg/L for PCT, 50 mg/L for CRP, 18×10^9/L for WBC, and 8×10^9/L for ANC. For assessment of *Mp*-positive CAP, the positive likelihood ratios (LR+) were highest for age >5 years (2.2, CI 1.4–3.3), duration of fever >6 days (3.1, CI 1.6–5.9) and RTI symptoms >6 days (2.5, CI 1.4–4.3), as well as for PCT <0.25 µg/L (2.2, CI 1.5–3.2) and CRP <50 mg/L (2.3, CI 1.4–3.5) **(Table 4)**. Using a PCT cut-off of <0.25 µg/L, only one (3%) *Mp*-positive patient had a PCT concentration above this cut-off compared with 19 (56%) *Mp*-negative patients ($p<0.01$). The *Mp*-positive CAP patient with the high PCT level (i.e., 0.55 µg/L) developed bronchiolitis obliterans and dermatological manifestations.

The combination of clinical features and biomarkers was more effective in the diagnosis of *Mp* CAP compared with either clinical features or biomarkers alone **(Figure 2C)**. Improved diagnostic performance was reached by combining clinical features (i.e., age with duration of prodromal symptoms, AUC=0.82, CI 0.7–0.9) together with PCT (AUC=0.90, CI 0.8–1.0, $p=0.05$) **(Table 4)**.

Discussion

We evaluated clinical features and biomarkers to diagnose *Mp* infection within a well-defined cohort of ambulatory and hospitalized children with CAP, in which *Mp* infection was diagnosed with the *Mp* IgM ASC ELISpot assay that allows for the differentiation between *Mp* infection and carriage [9]. Diagnosing *Mp* as the cause of CAP at an early stage is important in order to avoid ineffective first-line empirical β-lactam antibiotics and to evaluate targeted treatment against *Mp* in severe cases [2].

Several scores, ratios, algorithms, and prediction rules have been reported to diagnose *Mp* infection on the basis of clinical features [5, 7, 23, 24]. However, previous studies found no reliable signs or symptoms to differentiate *Mp* infection in CAP from other etiology [5, 6], potentially because *Mp* infection was misclassified as infection when it was carriage. We recently demonstrated that the *Mp* IgM ASC ELISpot assay differentiates *Mp* infection from carriage [9]: while *Mp* DNA and/or IgM was also detected in 48% and 29% healthy control children (*n*=21), all were tested negative by the *Mp* IgM ASC ELISpot assay [9]. Notably, the high *Mp* detection rate in CAP patients (46%) in this study may be related to the inclusion age of 3–18 years, in which *Mp* is most frequently detected [1, 2], and the coinciding *Mp* epidemic in Europe during the study period [25-28]. Prevalence estimates are important for translation of diagnostic study findings into clinical practice. When taking Bayes theorem into account, the post-test probability of disease presence will be higher given a higher pre-test probability or prevalence [29-31]. For proper implementation of a new diagnostic test into clinical practice it is therefore necessary that the test will be assessed in the context of all the other diagnostic information that is available at the time point of testing.

We were able to corroborate previous study findings in that *Mp*-positive CAP manifests predominantly in school-aged children >5 years [1, 12, 23] and children present with prodromal fever and respiratory symptoms of >6 days [1], significantly longer compared with CAP caused by other etiology. Other symptoms and signs, as well as radiographic findings did not differentiate *Mp*-positive from *Mp*-negative CAP [1, 5, 6]. Extrapulmonary manifestations, predominantly skin disorders, were statistically associated with *Mp*-positive CAP in this study. Its presence significantly increases the probability of underlying *Mp* infection [32]. This may be also true for the

presence of RTI within families of *Mp*-positive CAP patients. Our observation of frequent RTI symptoms and *Mp* detection in members of those families supports previous data about *Mp* spread among persons in close contact and family transmission of *Mp* [33-36].

Mp infection is mild and self-limiting in most cases, and manifests predominantly in previously healthy children [5]. In our study, one third of *Mp*-positive CAP patients were not treated with an antibiotic *in vitro* active against *Mp*, but all of these children fully recovered. The BTS guidelines advise that macrolide antibiotics may be added at any age in case of very severe disease or if there is no response to first-line empirical treatment [2]. Prior antibiotic treatment was associated with *Mp* CAP and included treatment with β-lactam antibiotics in most cases. This supports that in patients that do not respond to β-lactam antibiotics, *Mp* CAP should be considered [2, 7]. We did not find statistical differences in fever duration following CAP diagnosis, LOS, or recovery at follow-up between *Mp*-positive patients who did and did not receive antibiotics against *Mp* (data not shown). These findings support the need for future interventional studies assessing the effect of antibiotics for *Mp* CAP [1, 9, 37, 38].

Our study demonstrated that in *Mp*-positive CAP biomarker levels of PCT, CRP, WBC, and ANC were statistically lower compared with *Mp*-negative CAP. Despite limited numbers, this was even true for PCT and CRP of *Mp*-positive CAP in comparison to viral CAP (RSV) as a subgroup of *Mp*-negative CAP. Similar trends have been observed in previous CAP studies for CRP, WBC, and ANC [23, 39-43], and recently for PCT with median levels from 0.05–0.19 µg/L in CAP considered to be caused by *Mp* [39-41, 44]. A study with conflicting results suggesting higher PCT

levels associated with *Mp* may be hampered by diagnostics [45]. A PCT cut-off of <0.25 µg/L reached the best discriminatory power in differentiating *Mp*-positive from *Mp*-negative children with CAP, which is supported by previous studies [39-41, 44]. Only one *Mp*-positive patient had a PCT level of 0.55 µg/L and was above this cut-off.

Our study has several limitations. First, though sampling was performed in a relevant population of clinical CAP [9], the study population is small and represents a convenience sample from an observational study, and we cannot rule out that unintended selection occurred. However, even though the sample size is small for prediction, the scope of this study was to describe risk factors for *Mp* infection. Second, mild cases may not have been referred to our tertiary center. Third, the study enrolled children from 3–18 years to reduce the probability of viral infection [9]. However, younger children with *Mp* infection may have more likely other RTIs than CAP [43].

In conclusion, improved diagnostics helped to identify clinical features and biomarkers that may predict *Mp* CAP in children, such as age >5 years, no underlying disease, family with RTI, prior antibiotic treatment, prodromal respiratory symptoms and fever >6 days, as well as extrapulmonary (skin) manifestations. Biomarkers showed some differences between *Mp*-positive and *Mp*-negative patients in that *Mp*-positive was associated with low levels of CRP, WBC, ANC, and PCT, particularly a PCT cut-off of <0.25 µg/L. However, the study size is small and even the best combinations of clinical features and biomarkers may not be reliable enough to be used as a diagnostic itself. Our data support that a combination of clinical features and biomarkers may help physicians in identifying patients at high risk for

Mp CAP, which warrants further investigations in a larger cohort of children with *Mp* infection.

Author contributions:

P.M.M.S. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: P.M.M.S., and approved by all authors; **Acquisition of data:** P.M.M.S., S.K., M.S., P.P., C.R., C.K., T.H., C.G.; **Analysis and interpretation of data:** P.M.M.S., L.A., L.M.B., A.M.C.v.R., C.B.; **Drafting of the manuscript:** P.M.M.S.; **Critical revision of the manuscript for important intellectual content:** all authors; **Statistical analysis:** P.M.M.S., L.A., L.M.B.; **Obtained funding:** P.M.M.S.; **Administrative, technical, or material support:** P.M.M.S., M.S., C.K., T.H., C.G., C.B.

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Potential conflict of interest:

Nothing to disclose.

References

1 Kutty PK, Jain S, Taylor TH, et al. *Mycoplasma pneumoniae* among children hospitalized with community-acquired pneumonia. Clin Infect Dis **2019**; 1:5-12.

2 Harris M, Clark J, Coote N, et al. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax **2011**; ii1-23.

3 Meyer Sauteur PM. Challenges and progress toward determining pneumonia etiology. Clin Infect Dis **2019**; *in press*.

4 Spuesens EB, Fraaij PL, Visser EG, et al. Carriage of *Mycoplasma pneumoniae* in the upper respiratory tract of symptomatic and asymptomatic children: an observational study. PLoS Med **2013**; 5:e1001444.

5 Waites KB, Xiao L, Liu Y, Balish MF and Atkinson TP. *Mycoplasma pneumoniae* from the respiratory tract and beyond. Clin Microbiol Rev **2017**; 3:747-809.

6 Wang K, Gill P, Perera R, Thomson A, Mant D and Harnden A. Clinical symptoms and signs for the diagnosis of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia. Cochrane Database Syst Rev **2012**; CD009175.

7 Meyer Sauteur PM, Unger WWJ, van Rossum AMC and Berger C. The art and science of diagnosing *Mycoplasma pneumoniae* infection. Pediatr Infect Dis J **2018**; 11:1192-5.

8 Okada T, Morozumi M, Tajima T, et al. Rapid effectiveness of minocycline or doxycycline against macrolide-resistant *Mycoplasma pneumoniae* infection in a 2011 outbreak among Japanese children. Clin Infect Dis **2012**; 12:1642-9.

9 Meyer Sauteur PM, Seiler M, Truck J, et al. Diagnosis of *Mycoplasma pneumoniae* childhood pneumonia with measurement of specific antibody-secreting cells. Am J Respir Crit Care Med **2019**; *in press*.

10 British Thoracic Society of Standards of Care Committee. British Thoracic Society guidelines for the management of community-acquired pneumonia in childhood. Thorax **2002**; i1-24.

11 Cilla G, Onate E, Perez-Yarza EG, Montes M, Vicente D and Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol **2008**; 10:1843-9.

12 Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med **2015**; 9:835-45.

13 Rhedin S, Lindstrand A, Hjelmgren A, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. Thorax **2015**; 9:847-53.

14 Hardegger D, Nadal D, Bossart W, Altwegg M and Dutly F. Rapid detection of *Mycoplasma pneumoniae* in clinical samples by real-time PCR. J Microbiol Methods **2000**; 1:45-51.

15 Greiner O, Day PJ, Bosshard PP, Imeri F, Altwegg M and Nadal D. Quantitative detection of *Streptococcus pneumoniae* in nasopharyngeal secretions by real-time PCR. J Clin Microbiol **2001**; 9:3129-34.

16 Pneumonia Etiology Research for Child Health (PERCH) Study Group. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. Lancet **2019**; 10200:757-79.

17 Nijhuis RHT, Guerendiain D, Claas ECJ and Templeton KE. Comparison of ePlex respiratory pathogen panel with laboratory-developed real-time PCR

assays for detection of respiratory pathogens. J Clin Microbiol **2017**; 6:1938-45.

18 Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. Bulletin of the World Health Organization **2005**; 5:353-9.

19 Fancourt N, Deloria Knoll M, Barger-Kamate B, et al. Standardized interpretation of chest radiographs in cases of pediatric pneumonia from the PERCH study. Clin Infect Dis **2017**; suppl_3:S253-S61.

20 Katan M, Moon Y, von Eckardstein A, et al. Procalcitonin and Midregional Proatrial Natriuretic Peptide as Biomarkers of Subclinical Cerebrovascular Damage: The Northern Manhattan Study. Stroke **2017**; 3:604-10.

21 Fan J, Upadhye S and Worster A. Understanding receiver operating characteristic (ROC) curves. CJEM **2006**; 1:19-20.

22 R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. **2019**; http://www.R-project.org/; Accessed at September 13, 2019.

23 Fischer JE, Steiner F, Zucol F, et al. Use of simple heuristics to target macrolide prescription in children with community-acquired pneumonia. Arch Pediatr Adolesc Med **2002**; 10:1005-8.

24 Neeser OL, Vukajlovic T, Felder L, et al. A high C-reactive protein/procalcitonin ratio predicts *Mycoplasma pneumoniae* infection. Clin Chem Lab Med **2019**;

25 Gullsby K, Olsen B and Bondeson K. Molecular typing of *Mycoplasma pneumoniae* strains in Sweden from 1996 to 2017 and the emergence of a new P1 cytadhesin gene, variant 2e. J Clin Microbiol **2019**; 6:*published online*.

26 Kurkela S, Puolakkainen M, Hokynar K, et al. *Mycoplasma pneumoniae* outbreak, Southeastern Finland, 2017-2018: molecular epidemiology and laboratory diagnostic lessons. Eur J Clin Microbiol Infect Dis **2019**; *published online*.

27 Public Health England. Annual summary of respiratory *Mycoplasma pneumoniae* laboratory surveillance data, England and Wales, 2017. **2018**; Accessed September 13, 2019.

28 National Institute for Public Health and the Environment (RIVM). Annual report surveillance of influenza and other respiratory infections in the Netherlands: winter 2017/2018. **2018**; Accessed at September 13, 2019.

29 Bachmann LM, ter Riet G, Weber WE and Kessels AG. Multivariable adjustments counteract spectrum and test review bias in accuracy studies. J Clin Epidemiol **2009**; 4:357-61 e2.

30 Fischer JE, Bachmann LM and Jaeschke R. A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis. Intensive Care Med **2003**; 7:1043-51.

31 Leeflang MM, Rutjes AW, Reitsma JB, Hooft L and Bossuyt PM. Variation of a test's sensitivity and specificity with disease prevalence. CMAJ **2013**; 11:E537-44.

32 Meyer Sauteur PM, Theiler M, Büttcher M, Seiler M, Weibel L and Berger C. *Mycoplasma pneumoniae*-induced mucocutaneous disease: a prospective longitudinal cohort study. JAMA Dermatol **2019**; *in press*.

33 Dorigo-Zetsma JW, Wilbrink B, van der Nat H, Bartelds AI, Heijnen ML and Dankert J. Results of molecular detection of *Mycoplasma pneumoniae* among patients with acute respiratory infection and in their household contacts reveals children as human reservoirs. J Infect Dis **2001**; 4:675-8.

34 Walter ND, Grant GB, Bandy U, et al. Community outbreak of *Mycoplasma pneumoniae* infection: school-based cluster of neurologic disease associated with household transmission of respiratory illness. J Infect Dis **2008**; 9:1365-74.

35 Kannan TR, Hardy RD, Coalson JJ, et al. Fatal outcomes in family transmission of *Mycoplasma pneumoniae*. Clin Infect Dis **2012**; 2:225-31.

36 Suzuki Y, Seto J, Shimotai Y, et al. Polyclonal spread of multiple genotypes of *Mycoplasma pneumoniae* in semi-closed settings in Yamagata, Japan. J Med Microbiol **2019**; 5:785-90.

37 Biondi E, McCulloh R, Alverson B, Klein A and Dixon A. Treatment of mycoplasma pneumonia: a systematic review. Pediatrics **2014**; 6:1081-90.

38 Gardiner SJ, Gavranich JB and Chang AB. Antibiotics for community-acquired lower respiratory tract infections secondary to *Mycoplasma pneumoniae* in children. Cochrane Database Syst Rev **2015**; CD004875.

39 Wallihan RG, Suarez NM, Cohen DM, et al. Molecular distance to health transcriptional score and disease severity in children hospitalized with community-acquired pneumonia. Front Cell Infect Microbiol **2018**; 382.

40 Jeong JE, Soh JE, Kwak JH, et al. Increased procalcitonin level is a risk factor for prolonged fever in children with *Mycoplasma* pneumonia. Korean J Pediatr **2018**; 8:258-63.

41 Alcoba G, Keitel K, Maspoli V, et al. A three-step diagnosis of pediatric pneumonia at the emergency department using clinical predictors, C-reactive protein, and pneumococcal PCR. Eur J Pediatr **2017**; 6:815-24.

42 Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics **2004**; 4:701-7.

43 Gordon O, Oster Y, Michael-Gayego A, et al. The clinical presentation of pediatric *Mycoplasma pneumoniae* infections--A single center cohort. Pediatr Infect Dis J **2019**; 7:698-705.

44 Stockmann C, Ampofo K, Killpack J, et al. Procalcitonin accurately identifies hospitalized children with low risk of bacterial community-acquired pneumonia. J Pediatric Infect Dis Soc **2018**; 1:46-53.

45 Moulin F, Raymond J, Lorrot M, et al. Procalcitonin in children admitted to hospital with community acquired pneumonia. Arch Dis Child **2001**; 4:332-6.

46 Herklotz R, Luthi U, Ottiger C and Huber AR. [Metaanalysis of reference values in hematology]. Ther Umsch **2006**; 1:5-24.

Tables

Table 1. Demographic, epidemiological, and clinical characteristics of *Mp*-positive CAP vs. *Mp*-negative CAP in children.

	***Mp* + CAP**[1] (*n*=29)	***Mp* – CAP**[1] (*n*=34)	**OR (95% CI)**	***p***
Demographic characteristics				
Age (years)[2], median (IQR)	8.6 (6.3–11.0)	4.7 (3.9–6.2)	–	**<0.01**
Sex (male), *n* (%)	16 (55)	23 (68)	0.6 (0.2–1.9)	0.44
Season at enrollment, *n* (%):				
- Spring (March–May)	5 (17)	6 (18)	1.0 (0.2–4.4)	1.00
- Summer (June–August)	9 (31)	4 (12)	3.3 (0.8–16.8)	0.07
- Autumn (September–November)	9 (31)	8 (23)	1.5 (0.4–5.2)	0.58
- Winter (December–February)	6 (21)	16 (47)	0.3 (0.1–1.0)	**0.04**
Day-care or pre-/school attendance, *n* (%)	29 (100)	34 (100)	NA	1.00
Immunizations[3], *n* (%)	21/23 (91)	31/32 (97)	0.3 (0.0–7.0)	0.57
Underlying disease[4], *n* (%)	1 (3)	9 (26)	0.1 (0.0–0.8)	**0.02**
- Asthma or history of wheezing, *n*	1	1		
- Cardiovascular, *n*	0	0		
- Gastrointestinal, *n*	0	2		
- Neurological, *n*	0	2		
- Other, *n*	0	4		
Family with RTI, *n* (%)	17 (59)	10 (29)	3.3 (1.1–11.0)	**0.02**
- Mothers, *n*	14	4		
- Fathers, *n*	6	1		
- Siblings, *n*	17	7		
Family members with *Mp* detection in URT, *n* (%)	17/47 (36)	2/37 (5)	9.7 (2.0–93.2)	**<0.01**
- Mothers, *n* (%)	7/20 (35)	2/26 (8)	6.2 (1.0–69.7)	**0.03**
- Fathers, *n* (%)	1/13 (8)	0/10 (0)	NA	1.00
- Siblings, *n* (%)	9/14 (64)	0/1 (0)	NA	0.40
- Symptomatic (RTI), *n* (%)	10/17 (59)	1/2 (50)	1.4 (0.0–123.1)	1.00
- Asymptomatic (carrier), *n* (%)	7/17 (41)[5]	1/2 (50)[5]	0.7 (0.0–62.7)	1.00
Prior antibiotic treatment, *n* (%)	13 (45)	5 (15)	4.6 (1.3–19.6)	**0.01**
Clinical presentation				
Prodrome:				
- RTI symptoms (days), median (IQR)	9.0 (6.0–10.0)	4.0 (3.0–7.0)	–	**<0.01**
- Fever (days), median (IQR)	8.0 (6.0–10.0)	4.0 (3.3–6.0)	–	**<0.01**
- Fever >2 days[6], *n* (%)	26 (90)	28 (82)	1.8 (0.3–12.5)	0.49
Fever (°C) at presentation[6], median (IQR)	39.1 (39.0–39.7)	39.2 (39.0–39.5)	–	0.89
RTI symptoms and signs at presentation[7]:				
- Runny nose, *n* (%)	7 (24)	14 (41)	0.5 (0.1–1.5)	0.19
- Sore throat, *n* (%)	4 (14)	4 (12)	1.2 (0.2–7.1)	1.00
- Cough, *n* (%)	27 (93)	30 (88)	1.8 (0.2–21.2)	0.68
- Chest pain, *n* (%)	3 (10)	7 (21)	0.5 (0.1–2.2)	0.32
- Wheezing, *n* (%)	0 (0)	1 (3)	NA	1.00
- Abnormal auscultatory findings, *n* (%)	19 (66)	24 (71)	0.8 (0.2–2.6)	0.79
- Oxygen saturation <93%, *n* (%)	5 (17)	8 (24)	0.7 (0.2–2.8)	0.76
Radiographic findings				
Pulmonary infiltrate in chest radiograph[8], *n* (%)	28/28 (100)	31/32 (97)	NA	1.00
Consolidation, *n* (%)	18 (64)	25 (78)	0.5 (0.1–1.8)	0.26
- Single lobar infiltrate, *n*	13	16		
- Multilobar infiltrates, *n*	5	9		
- Multilobar infiltrates (unilateral), *n*	1	3		
- Multilobar infiltrates (bilateral), *n*	4	6		
Interstitial, *n* (%)	10 (36)	6 (19)	2.4 (0.6–9.5)	0.16
- Reticular, *n*	10	6		
- Nodular, *n*	0	0		
Pleural effusion, *n* (%)	4 (14)	7 (22)	0.6 (0.1–2.7)	0.52

Severity of illness				
Hospitalization, *n* (%)	10 (34)	19 (56)	0.4 (0.1–1.3)	0.13
- LOS (days), median (IQR)	4.5 (3.3–7.0)	2.5 (2.0–5.8)	–	0.31
- ICU admission, *n* (%)	0 (0)	1 (3)	NA	1.00
Extrapulmonary manifestation, *n* (%)	9 (31)[9]	0 (0)	NA	**<0.01**
- Dermatological, *n*	8			
- Neurological, *n*	1			
Treatment				
Antibiotics after enrollment, *n* (%)	27 (93)[10]	31 (91)[10]	1.3 (0.1–16.7)	1.00
- Co-/amoxicillin, *n* (%)	13 (45)	29 (85)	0.1 (0.0–0.5)	**<0.01**
- Clarithromycin, *n* (%)	10 (34)	1 (3)	16.7 (2.1–772.7)	**<0.01**
- Doxycycline, *n* (%)	10 (34)	1 (3)	16.7 (2.1–772.7)	**<0.01**
- Other, *n* (%)	1 (3)	2 (6)	0.6 (0.0–11.6)	1.00
Outcome				
Full recovery, *n* (%)	26 (90)[11]	34 (100)	NA	0.09
- Respiratory sequelae, *n*	2			
- Dermatological sequelae, *n*	1			

Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; Co-/amoxicillin, amoxicillin with or without clavulanic acid; IQR, interquartile range; ICU, intensive care unit; LOS, length of hospital stay; *Mp*, *Mycoplasma pneumoniae*; NA, not available; OR, odds ratio; RTI, respiratory tract infection; URT, upper respiratory tract.

Differences between groups were determined by the Mann-Whitney *U* test (medians) and Fisher's exact test (proportions).

[1] Defined according to positive *Mp*-specific IgM ASC ELISpot assay results [9]: *Mp* + CAP: All 29 patients were *Mp* IgM ASC-positive and were also *Mp* PCR-positive and *Mp* IgM-seropositive; *Mp* – CAP: All 34 patients were *Mp* IgM ASC-negative and were also *Mp* IgM-seronegative. Three patients who tested negative for *Mp* IgM ASCs were *Mp* PCR-positive. Another pathogen was found based on the results of multiplex PCR from pharyngeal swab samples and specific serology that may have caused CAP in those cases [9];

[2] Only patients between 3 and 18 years were enrolled (inclusion criteria) [9];

[3] ''Immunizations'' refers to being immunized per the national immunization schedule in Switzerland;

[4] Chronic lung disorders (excluding asthma) were part of the exclusion criteria [9];

[5] Asymptomatic carriers: *Mp* + CAP: *n*=6 siblings, *n*=1 mother; *Mp* – CAP: *n*=1 mother;

[6] According to the prediction rule for risk of *Mp* infection in children with CAP by Fischer et al. [23];

[7] Inclusion criteria were clinical diagnosis of CAP with fever >38.5°C and tachypnea according to the BTS guidelines [2];

[8] Chest radiographs were not part of inclusion criteria, but routinely performed in 60 out of 63 (95%) included CAP patients; 98% (*n*=59/60) met the criteria for radiological pneumonia [9];

[9] Dermatological (*n*=8) [32]: *Mp*-induced rash and mucositis (MIRM) (*n*=3), urticaria (*n*=2), and maculopapular skin eruptions (*n*=3); neurological (*n*=1): aseptic meningitis;

[10] Antibiotic treatment with ≥1 agent: *Mp* + CAP: co-/amoxicillin + clarithromycin (*n*=4), co-/amoxicillin + doxycycline (*n*=3); *Mp* – CAP: amoxicillin + clarithromycin (*n*=1), co-/amoxicillin + ceftazidime (*n*=1). *Mp* + CAP not treated with an antibiotic *in vitro* active against *Mp*: *n*=9 (31%); all of them fully recovered;

[11] Abnormal outcomes: *Mp* + CAP: bronchiolitis obliterans with decreased lung function (*n*=1), exertional dyspnea without physical findings, i.e., normal lung and cardiac function (*n*=1), post-inflammatory pigmentary alteration (*n*=1).

Table 2. Laboratory findings of *Mp*-positive CAP vs. *Mp*-negative CAP in children.

	***Mp* + CAP** (*n*=29)	***Mp* – CAP** (*n*=34)	**OR (95% CI)**	***p***
Blood cell count		9		
WBC (×10^9/L), median (IQR)	8.97 (6.91–11.80)	12.30 (9.11–20.48)	–	**0.01**
- Abnormal[1], *n* (%)	5/21 (24)	13/26 (50)	0.3 (0.1–1.3)	0.08
- Leukocytosis, *n*	4	12		
- Leukopenia, *n*	1	1		
ANC (×10^9/L), median (IQR)	6.63 (4.21–8.70)	10.00 (6.17–17.29)	–	**0.02**
- Abnormal[1], *n* (%)	7/21 (33)	14/25 (56)	0.4 (0.1–1.5)	0.15
- Neutrophilia, *n*	7	14		
- Neutropenia, *n*	0	0		
Lymphocytes (×10^9/L), median (IQR)	1.45 (1.16–2.29)	1.77 (1.19–2.58)	–	0.52
- Abnormal[1], *n* (%)	8/21 (38)	7/24 (29)	1.5 (0.4–6.2)	0.55
- Lymphocytosis, *n*	0	0		
- Lymphopenia, *n*	8	7		
Monocytes (×10^9/L), median (IQR)	0.71 (0.47–0.96)	0.89 (0.63–1.11)	–	0.21
- Abnormal[1], *n* (%)	2/21 (10)	1/24 (4)	2.4 (0.1–148.8)	0.59
- Monocytosis, *n*	2	1		
- Absolute monocytopenia, *n*	0	0		
Eosinophils (×10^9/L), median (IQR)	0.13 (0.04–0.22)	0.01 (0.00–0.13)	–	0.05
- Abnormal[1], *n* (%)	3/20 (15)	10/24 (42)	0.3 (0.0–1.3)	0.09
- Eosinophilia, *n*	1	2		
- Absolute eosinopenia, *n*	2	8		
Basophils (×10^9/L), median (IQR)	0.05 (0.02–0.07)	0.04 (0.02–0.07)	–	0.98
- Abnormal[1], *n* (%)	0/20 (0)	0/24 (0)	NA	1.00
Anemia[1], *n* (%)	2/21 (10)	8/26 (31)	0.2 (0.0–1.5)	0.15
Platelet count (×10^9/L), median (IQR)	315 (289–378)	310 (252–356)	–	0.27
- Abnormal[1], *n* (%)	6/21 (29)	4/25 (16)	2.1 (0.4–11.8)	0.48
- Thrombocytopenia, *n*	5	3		
- Thrombocytosis, *n*	1	1		
Chemistry				
PCT (µg/L), median (IQR)	0.06 (0.04–0.14)	0.28 (0.12–1.75)	–	**<0.01**
CRP (mg/L), median (IQR)	16 (8–36)	72 (24–170)	–	**<0.01**
CRP/PCT ratio (mg/µg), median (IQR)	200 (84–452)	104 (39–320)	–	0.27
CRP/PCT ratio >400 mg/µg[2], *n* (%)	9 (31)	7 (21)	1.7 (0.5–6.5)	0.39

Abbreviations: ANC, absolute neutrophil count; CAP, community-acquired pneumonia; CI, confidence interval; CRP, C-reactive protein; IQR, interquartile range; *Mp*, *Mycoplasma pneumoniae*; NA, not available; OR, odds ratio; PCT, procalcitonin; WBC, white blood cell count.

Differences between groups were determined by the Mann-Whitney *U* test (medians) and Fisher's exact test (proportions).

[1] Reference values for hematology were defined as previously described [46];
[2] According to the admission CRP/PCT ratio for risk of *Mp* infection in hospitalized adults with CAP by Neeser et al. [24].

Table 3. Pathogen detection in URT of *Mp*-positive CAP vs. *Mp*-negative CAP in children.

	***Mp* + CAP** (*n*=29)	***Mp* – CAP** (*n*=34)	**OR (95% CI)**	***p***
Pathogen				
Any pathogen, *n* (%)	29 (100)	29 (85)	NA	0.06
≥2 pathogens, *n* (%)	20 (69)	13 (38)	3.5 (1.1–11.7)	**0.02**
≥3 pathogens, *n* (%)	7 (24)	5 (15)	1.8 (0.4–8.4)	0.52
Bacteria				
Any bacteria, *n* (%)	29 (100)	26 (76)	NA	**<0.01**
Mp, *n* (%)	29 (100)	3 (9)	NA	**<0.01**
Sp, *n* (%)	18 (62)	26 (76)[1]	0.5 (0.1–1.7)	0.27
Chlamydophila pneumoniae, *n* (%)	0 (0)	1 (3)	NA	1.00
Legionella pneumophila, *n* (%)	0 (0)	0 (0)	NA	1.00
Bordetella pertussis, *n* (%)	0 (0)	0 (0)	NA	1.00
Viruses				
Any virus, *n* (%)	9 (32)	15 (45)	0.6 (0.2–1.8)	0.31
RSV, *n* (%)	0 (0)	8 (24)[2]	NA	**<0.01**
- A		2		
- B		3		
Influenza virus, *n* (%)	0 (0)	2 (6)	NA	0.50
- A		2		
- H1		0		
- H1-2009		0		
- H3		1		
- B		0		
Parainfluenza virus, *n* (%)	0 (0)	2 (6)	NA	0.50
- 1		0		
- 2		2		
- 3		0		
- 4		0		
Human rhinovirus/enterovirus[3], *n* (%)	3 (11)	4 (12)	0.9 (0.1–5.7)	1.00
Adenovirus, *n* (%)	5 (18)	3 (9)	2.1 (0.4–15.3)	0.45
Human metapneumovirus, *n* (%)	0 (0)	0 (0)	NA	1.00
Coronavirus, *n* (%)	0 (0)	1 (3)	NA	1.00
- 229E		0		
- HKU1		0		
- NL63		0		
- OC43		1		
- MERS coronavirus		0		
Human bocavirus, *n* (%)	1 (4)	1 (3)	1.2 (0.0–95.8)	1.00

Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; IQR, interquartile range; MERS, Middle East respiratory syndrome; *Mp*, *Mycoplasma pneumoniae*; NA, not available; OR, odds ratio; RSV, respiratory syncytial virus; *Sp*, *Streptococcus pneumoniae*.

Pharyngeal samples were tested in all patients by real-time PCR for *Mp* [14] and *Sp* [15], and for other bacterial and viral pathogens by the ePlex respiratory panel [17] in 28 of 29 *Mp* + CAP and 33 of 34 *Mp* – CAP patients with residual respiratory samples. Blood cultures were performed in 14 (48%) *Mp* + CAP and 20 (59%) *Mp* – CAP patients during routine clinical care and all were negative. Routine PCR testing for *Mp* [14] as part of clinical care was positive in *n*=8/8 *Mp* + CAP and *n*=0/11 *Mp* – CAP patients.

Differences between groups were determined by the Mann-Whitney *U* test (medians) and Fisher's exact test (proportions).

[1] Two patients had *Sp* detected in both the URT and pleural fluid samples (pleural puncture performed during routine clinical care). They had no other pathogens detected in the URT apart from *Sp*;

[2] RSV was additionally detected in nasopharyngeal samples of three patients by rapid antigen detection test (Quidel Sofia, San Diego, CA, USA) during routine clinical care;

[3] No differentiation possible between rhinovirus and enterovirus [17].

Table 4. Best thresholds with diagnostic accuracy for *Mp*-positive CAP vs. *Mp*-negative CAP in children.

Parameter	Threshold	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR– (95% CI)	AUC (95% CI)
Clinical features						
Age (years)	**>5**	0.90 (0.74–0.96)	0.59 (0.42–0.74)	2.18 (1.43–3.31)	0.18 (0.06–0.53)	0.77 (0.64–0.89)
Fever (days)	**>6**	0.72 (0.54–0.85)	0.76 (0.60–0.88)	3.08 (1.61–5.87)	0.36 (0.19–0.67)	0.72 (0.59–0.86)
RTI symptoms (days)	**>6**	0.72 (0.54–0.85)	0.71 (0.54–0.83)	2.46 (1.40–4.34)	0.39 (0.21–0.73)	0.79 (0.68–0.91)
Biomarkers						
PCT (µg/L)	**<0.25**	0.97 (0.83–0.99)	0.56 (0.39–0.71)	2.19 (1.49–3.21)	0.06 (0.01–0.43)	0.81 (0.70–0.92)
CRP (mg/L)	**<50**	0.86 (0.69–0.95)	0.62 (0.45–0.76)	2.25 (1.44–3.54)	0.22 (0.09–0.58)	0.76 (0.63–0.88)
WBC ($\times 10^9$/L)	**<18.00**	0.95 (0.77–0.99)	0.38 (0.22–0.57)	1.55 (1.13–2.13)	0.12 (0.02–0.89)	0.71 (0.56–0.86)
ANC ($\times 10^9$/L)	**<8.00**	0.67 (0.45–0.83)	0.60 (0.41–0.77)	1.67 (0.94–2.94)	0.56 (0.28–1.10)	0.71 (0.56–0.86)
Combination						
Age + fever + RTI symptoms	as defined above	1.00 (0.88–1.00)	0.44 (0.29–0.61)	1.79 (1.33–2.41)	NA	0.82 (0.71–0.92)
Age + fever + RTI symptoms + PCT	as defined above	1.00 (0.88–1.00)	0.32 (0.19–0.49)	1.48 (1.17–1.87)	NA	0.90 (0.83–0.97)

Abbreviations: ANC, absolute neutrophil count; AUC, area under the receiver operating characteristics curve; CAP, community-acquired pneumonia; CI, confidence interval; CRP, C-reactive protein; LR+, positive likelihood ratio; LR-, negative likelihood ratio; *Mp*, *Mycoplasma pneumoniae*; NA, not available; PCT, procalcitonin; RTI, respiratory tract infection; WBC, white blood cell count.

The threshold is the optimal cut-off that maximizes the distance to the identity (diagonal) line in the ROC curve in **Figure 2** according to Youden's J statistic using the "coords" function in R software environment (version 3.6.0) [22].

Figure legends

Figure 1. Biomarker test results of *Mp*-positive CAP vs. *Mp*-negative CAP in children. *A:* Procalcitonin (PCT). *B:* C-reactive protein (CRP). *C:* White blood cell count (WBC). *D:* Absolute neutrophil count (ANC). The *p* value is indicated in the graphs (Mann-Whitney *U* test). Abbreviations: CAP, community-acquired pneumonia; *Mp*, *Mycoplasma pneumoniae*.

Figure 2. Receiver operating characteristics (ROC) curve of sensitivity vs. specificity for clinical features (*A*), biomarkers (*B*), and a combination of both (*C*). Area under the ROC curve (AUC) differences (DeLong's test): *A*–*B*, no significant AUC differences; *C*, **p*=0.37 (age vs. combination of clinical features), ***p*=0.05 (combination of clinical features vs. combination of clinical features + PCT). Abbreviations: ANC, absolute neutrophil count; CRP, C-reactive protein; PCT, procalcitonin; RTI, respiratory tract infection; WBC, white blood cell count.

Figure 1

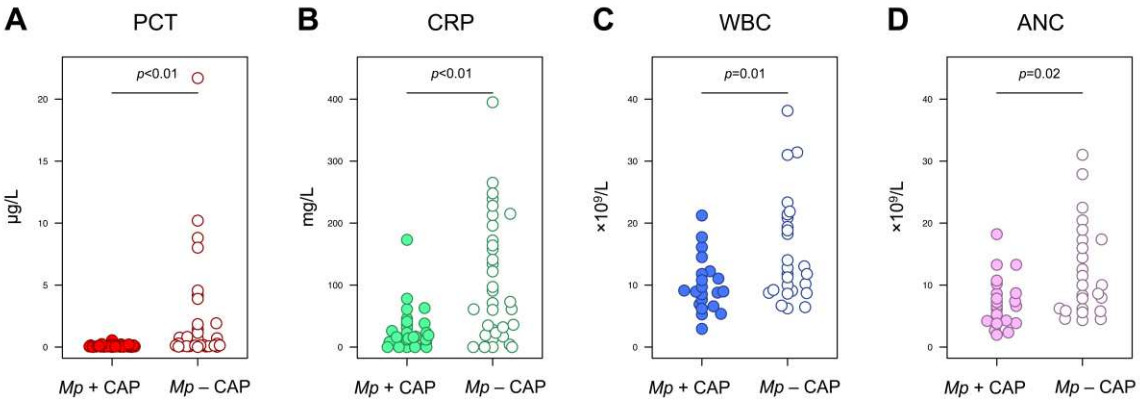

Figure 2